Effects of Combined Abiotic Stresses On Nutrient Content of European Wheat and Implications For Nutritional Security Under Climate Change Scenarios

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Abstract

Climate change is causing problems for agriculture, but the effect of combined environmental stresses on crop nutritional quality is not clear. Here we studied the effect of 10 combinations of climatic conditions (temperature, CO₂, O₃ and drought) in controlled growth chamber conditions on the protein and mineral content of 3 wheat varieties. Results show that wheat plants under O₃ exposure alone concentrated 15-31% more grain N, Fe, Mg, Mn P and Zn, reduced K by 5%, and C did not change. Ozone in the presence of elevated CO₂ and higher temperature enhanced the content of Fe, Mn, P and Zn by 2-18%. Water-limited chronic O₃ exposure resulted in 9-46% higher concentrations of all the minerals, except K. The effect of climate change could increase the ability of wheat to meet adult daily dietary requirements by 1.06-1.12-fold for Fe, Zn and protein, but decrease those of Mg, Mn and P by 1.03-1.06-fold, and K by 2.78-fold. The role of wheat in future nutrition security is discussed.

1. Main

Wheat (*Triticum aestivum* and *T. durum*) is the third most produced food crop worldwide after maize and rice, with 766 million tons harvested in 2019, mainly in Asia (44.1%), Europe (34.8%) and the Americas (15.3%). Wheat is key to food and nutrition security of populations worldwide. Average daily supply of wheat and its products is estimated at 179 g per capita, providing 527 kcal, 15.8 g of protein and 2.4 g of fats. It is consumed in all the continents, Europeans being the largest consumers with 298.6 g/day, followed by Oceanians, Asians, and Americans. Africans have the lowest consumption of 130.6 g/day¹. Among the world main staples, wheat shows high content of proteins (~13%), carbohydrates (~71%) and dietary fiber (~12%), and comparatively high amount of several minerals, the highest being manganese, selenium, calcium, zinc and copper². Despite the importance of wheat as human food, more than 800 million people are undernourished in the world, and between 1.5 to 2 billion people suffer from one or more chronic micronutrient deficiencies (MNDs), notably deficiencies in calcium, iodine, iron, selenium, zinc, folate and vitamin A³⁻⁶. Any change to the nutrient content of wheat can have a large impact on nutrient intake and dietary health of millions of people. To address global food security, sustainable crop production is crucial and requires a suitable input of chemical fertilizers containing macro and micronutrients. However, climatic factors beyond farmers' management capabilities determine the final crop yields. World's climate has been changing more rapidly than ever due to human activities such as industrialization and deforestation, and these changes can dramatically affect crop production and availability of nutrients for human consumption⁷. Water, temperature and CO₂ are well-known parameters influencing the growth and development of plants. Another climatic factor, the concentration of ozone (O₃), can exert synergistic or antagonistic interactions on plant defense mechanism and influence plant growth and yield in various ways⁸.

Individual climate factors can affect wheat production and grain quality in different ways. High temperature treatment (40/20 vs 25/20°C day/night) ten days after anthesis reduced grain number, weight, and the content of polysaccharides and proteins in many wheat varieties⁹. Elevated concentration of atmospheric CO₂ (546–586 ppm) lead to lower concentrations of Zn and Fe in C₃ grains and legumes, and C₃ crops other than legumes showed lower concentrations of protein¹⁰. Högy et al.¹¹ observed an increase of 1000-grain weight, but a decrease in proteins, amino acids, Fe and Ca when CO₂ concentration was 150 ppm above the ambient value. Similarly, concentrations of grain protein, Fe, Zn, S and Ca were significantly reduced at elevated CO₂ (550 ppm) as compared to the ambient 384 ppm¹². Chronic elevated O₃ (+25-35 ppb) resulted in lower rubisco enzyme activity, chlorophyll and photosynthetic rate in *T. aestivum* wheat, and significantly reduced sugar, starch and protein contents in the two wheat varieties¹³. A review of 42 experiments performed in Asia, Europe and North America showed that high O₃ has a strong negative effect on 1000-grain weight, and weaker but significant negative effects on starch concentration and volume weight. Conversely, significant increase of protein and several nutritionally important minerals (K, Mg, Ca, P, Zn, Mn, Cu) was observed, but yields were significantly decreased. For Fe, S and Na, effects were not significant or results were inconclusive¹⁴.

Climate change in the recent decades has led to significant geographical and seasonal redistribution in precipitation and rise in temperature¹⁵. Water deficit (drought), non-uniform precipitation, and the occurrence of prolonged dry spells, especially when happening in the critical stage of crop development, have a direct effect on the grain yield, physical and nutritional quality of crops worldwide. Therefore, it is necessary to obtain crops that under conditions of low water availability maintain high productive potential without losing their nutritional quality¹⁶. In wheat, water deficit at grain filling stage reduces grain-filling duration and ultimately reduces grain number and size¹⁷. Water deficit also reduced grain yield of common bean, triticale and wheat, reduced macro and micronutrient contents in the grains, caused an undesirable change in their physical quality, and altered their chemical quality¹⁸. Under severe drought conditions, a significant reduction in total protein and carbohydrates and a gradual augmentation in total fibers in wheat grains was observed¹⁹. Contrarily, moderate drought during grain filling in wheat was found to increase grain protein content, although a slight decrease in grain yield was also observed²⁰. However, the outcome of the combined effect of drought with other climate change factors on wheat grain quality is not yet well understood.

Simultaneous combinations of changes in climatic factors may not result in additive effects on plant growth and productivity²¹⁻²³. Wheat cultured under higher temperature and CO₂ conditions (700 ppm CO₂ and 3°C temperature rise) had significantly lower straw and grain yield, particularly due to severe reduction in number of spikes per plant, although supplied with ample fertilization⁷. Multifactor combination of ambient or elevated CO₂ (385 and 700 ppm), O₃ (20 and 60 ppb) and temperature (19/12 and 24/17°C) showed a decrease in growth and production in oilseed rape and barley²¹. In soybean however, high CO₂ (600 ppm) decreased Fe and Zn concentration in grains, while combination of high temperature (+3.4/+2.7°C) and high CO₂ restored the content of these minerals to levels obtained under ambient CO₂ (400 ppm) and canopy temperature conditions²⁴. In the opposite, maximum decrease in wheat grain yield (43.6%) was observed under the additive effect of 50% water deficit stress and elevated O₃ (+20 ppb), while water deficit stress alone reduced grain yield by 19.8%, and elevated O₃ alone by 17.9%²⁵. More interestingly, a climate chambers multifactorial experiment with combination of CO₂ (400 and 700 ppm), temperature (19/12 and 24/17°C) and different O₃ exposure regimes (5.9-7.2 ppb and episodic or chronic 80 to 100 ppb) on different wheat varieties showed that their growth responses and yields were affected differently by climate factor combinations, and response to temperature change overrode responses to

changes in CO₂ and O₃ ²⁶. These observations underline the complexity of plant response to changes in climatic factors, and therefore, when accessing the effects of future climatic conditions on crops and foods, multiple environmental parameters must be simultaneously considered.

Response to climate change differs not only among crop species ^{10,21,27}, but also among crop varieties of the same species. Elevated O₃ caused higher yield loss in normal maize cultivars than in quality protein maize (QPM) cultivars; carbohydrate content reduced more in normal maize, while essential amino acids and saturated fatty acids showed more decline in QPM ²⁸. Under elevated O₃, more reduction of grain yield was observed in *T. aestivum* (15 and 19%) as compared to *T. durum* (9 and 13%) ¹³. Similarly, the landrace variety of wheat was more sensitive to O₃ than the modern varieties ²⁶. These differences of response between cultivars of a single crop suggest that breeding could partly address the challenges of climate change to crop production ¹⁰, and this implies that different varieties should be assessed when measuring climate change effects on crops.

Without action, climate change will impact nutrition through decreased food quantity and access, decreased dietary diversity, and decreased food nutritional content ^{29,30}. Modelling studies have investigated how elevated CO₂ would impact nutritional quality of staples globally, notably its impact on protein ³¹, Zn ³², Fe ³³, and nutrients in general ³⁴. Changes in nutrient content of wheat as result of climate change may also influence the amount of wheat to be consumed in order to meet the nutrient needs of populations. In order to obtain a full picture of the threats of climate change to food security, a number of quality effects including yield of protein and important minerals need to be assessed, together with their direct potential adverse effects on malnutrition and health ¹⁴. Moreover, several authors recommended that studies should include interactions of global change parameters that might have strong effects on agriculture such as increase of O₃, elevated CO₂, rising temperatures and changes in water availability ^{14,27,35,36}. Very little consideration have been given to interaction of O₃ with other abiotic factors on yield and nutrient content of plants. Besides, to our knowledge, no attempts have been made to measure to what extend combined climate change factors will affect food consumption and dietary intake to fulfil the nutritional requirements of the population. Therefore, this study aimed to evaluate the effect of combinations of future climate scenarios (temperature, CO₂, O₃ and drought) on nutrient content of 3 spring wheat varieties, and estimate the consequence of changes in nutrient content on wheat contribution to nutrition security in a climate changing world.

2. Results

2.1. Effects of climate parameters on wheat mineral content

2.1.1. Carbon

The C content of wheat varieties in the controls was between 45.08 and 45.21 g/100g dw. Minor significant higher C content (between 0.47% and 0.16%) were obtained in some temperature treatments, but in general, the climate factors did not considerably affect C content of wheat varieties. Content of C in Lantvete was significantly higher than that of Lennox (p=.028), but did not differ from content in KWS Bittern (p=.412) (Figure 1A, Supplementary table 1 and Supplementary Figure 1).

2.1.2. Nitrogen, protein and gluten

The initial N content of wheat varieties ranged from 1.89 to 1.94 g/100g dw. Significantly higher concentrations were recorded in response to all the climate change treatments, in all the wheat varieties, from 3.99–51.92%. The varieties responded differently to each treatment: the highest N values in KWS Bittern (43.37% and 43.63%) were obtained with treatments CT.O3 and T.EpO3, respectively; in Lantvete it was 50.81% with treatment CT; while in Lennox it was 51.92% with CT.EpO3. In all the varieties, treatment A.EpO3 lead to the lowest N increase (Figure 1B).

When each climate parameter was considered alone, highest N values were obtained with chronic O₃ treatments, followed by episodic and then normal O₃; N content under high CO₂ treatments were slightly greater than under ambient CO₂; treatments with higher temperature showed higher N content than those with lower temperature. Under presence of O₃ however, high CO₂ equally affects N content irrespective of the O₃ regime, while for ambient CO₂, N content is higher with episodic O₃, and highest with chronic O₃. Similarly, higher temperature has a comparable effect on N content irrespective of the O₃ regime, while the effect of lower temperature is enhanced under episodic O₃, and more enhanced under choric O₃ (Supplementary Figure 2).

Water-limited treatment with KWS Bittern slightly reduced N content by 2.10–4.57%, when compared to their respective controls. But when compared to the control treatment (A), significant increase (p<.001) of 29.31% and 40.37% were obtained for treatments WLA.O3 and WLCT.O3, respectively (Supplementary Table 1).

Overall, for all the control and the treatments, N content of landrace variety Lantvete was significantly higher than that of modern varieties KWS Bittern (p=.013) and Lennox (p=.017) (Supplementary Table 1 and Supplementary Figure 2).

Protein content of wheat grains followed the same pattern as N content, as the former was mathematically derived from the later: it varied from 11.64 to 12.11 g/100g dw, all the climate change treatments resulted in significant higher protein content. The content of gluten in wheat grains of the controls was between 22.22% and 23.40% proteins. It varied with climate change treatments following the similar trend of protein content.

2.1.3. Iron

The initial content of Fe in wheat varieties was between 2.33 and 2.40 mg/100g dw. Climate change treatments significantly enhanced Fe concentration in grains, by 15.07–78.58% in Lantvete, 9.31–76.10% in KWS Bittern, and 18.46–59.50% in Lennox. A similar pattern was observed in all the varieties: the highest increased was recorded with treatment CT.O3 or CT.EpO3, followed by T.EpO3, A.O3 and CT; while C.EpO3 and A.EpO3 recorded the lowest Fe increase (Figure 1C).

Chronic O₃ treatments showed highest values of Fe content, followed by episodic O₃, and then normal O₃. Treatments with high CO₂ yielded higher Fe than those with ambient CO₂. Higher temperature treatments showed higher Fe contents than treatments at lower temperature. The effect of CO₂ was boosted by episodic and chronic O₃ exposure, as compared to the normal exposure. Similar observation was recorded on effect of temperature in presence of O₃ exposure (Supplementary Figure 3).

When compared to their respective controls, the water-limited treatment did not affect Fe content, but as compared to the control treatment (A), a significant increase ($p < .001$) of Fe content was obtained with treatment WLA.O3 (50.42%) and WLCT.O3 (68.60%) (Supplementary Table 1).

Landrace variety Lantvete showed significantly higher Fe content than modern varieties KWS Bittern ($p = .001$) and Lennox ($p = .023$) (Supplementary Table 1 and Supplementary Figure 3).

2.1.4. Potassium

Potassium content of the control treatments was in the range 525.99-606.84 mg/100g dw and significantly decreased by the treatments, in all the 3 varieties, with a reduction of up to 11.29% in KWS Bittern ($p < .001$) and 19.87% in Lennox ($p < .001$). An increase under some treatments, but non-significant, was recorded with variety Lantvete ($p = .130$). The reductions of K were observed in the higher CO₂ and O₃ treatments C.EpO₃, CT.EpO₃ and CT.O₃ in KWS Bittern, and CT.EpO₃ in Lennox (Figure 1D).

The different O₃ treatments had similar effect on K content (Figure 4B), and no clear difference could be observed on the effect of the combined treatments on K content.

Except a marginal significant decrease of 8.70% ($p = .008$) obtained with WLA.O3 against A.O3, K content was not affected by drought treatments (Supplementary Table 1).

On the other hand, K content of Lantvete was significantly lower than that of KWS Bittern ($p = .001$), but comparable to its content in Lennox ($p = .131$) (Supplementary Table 1 and Supplementary Figure 4).

2.1.5. Magnesium

The wheat varieties initially contained Mg between 109.39 and 141.22 mg/100g dw. It significantly increased with the climate change treatments, a 23.18–34.03% increase was recorded in Lantvete, between 8.65% and 29.71% in KWS Bittern, and between 18.73% and 52.46% in Lennox. Treatment C.EpO3 did not show any significant change of Mg content in all the three varieties. Except A.O3 in KWS Bittern and Lantvete, in general, the highest Mg increases were obtained with the higher temperature treatments CT, CT.EpO3, CT.O3 and T.EpO3 (Figure 1E).

Magnesium content of treatments with chronic O₃ exposure was higher than for treatment with episodic and normal O₃. Most of the treatments with high CO₂ showed higher Mg content than treatments with ambient CO₂. Higher temperature treatments resulted in Mg content higher than those of lower temperature. The effect of high CO₂ on Mg content was not affected by any the three O₃ exposure regimes, while the effect of ambient CO₂ was enhanced by episodic and chronic O₃. Similarly, the three O₃ regimes did not influence higher temperature effect on Mg content, but lower temperature showed a stronger effect under chronic O₃, followed by episodic, and normal O₃ (Supplementary Figure 5).

The water-limited treatments did not affect Mg content when compared to their respective controls, but when compared to the control treatment A, drought enhanced a significant increase ($p < .001$) of Mg content of 13.98% and 28.45% for treatments WLA.O3 and WLCT.O3, respectively (Supplementary Table 1).

Overall, the landrace variety Lantvete showed significantly higher Mg content than modern varieties KWS Bittern ($p < .001$) and Lennox ($p = .003$) (Supplementary Table 1 and Supplementary Figure 5).

2.1.6. Manganese

Manganese content of the control treatments was in the range 6.83-7.59 mg/100g dw. Some climate change treatments resulted in a significant increase of grain Mn by 40.43% (A.O3) in Lantvete, from 20.98% (C.EpO3) to 26.21% (T.EpO3) in Lennox, and 10.91% (A.EpO3) and 12.68 (A.O3) in KWS Bittern. The other treatments resulted in minor non-significantly higher or lower Mn content than in the control A (Figure 1F).

Taken individually, the treatments showing higher values of Mn content were chronic O₃ exposure, followed by episodic O₃, and then normal O₃, ambient CO₂ and lower temperature. Similarly, interaction of episodic O₃ exposure with high CO₂ or with higher temperature showed no effect, but chronic O₃ enhanced higher Mn content in the presence of ambient CO₂ or lower temperature (Supplementary Figure 6).

Treatments with deficiency of water lead to slightly significantly higher Mn contents than those of their respective controls (9.09% for WLA.O3 and 8.54% for WLCT.O3). Comparatively with control A, only treatment WLA.O3 resulted in a significant increase of 22.92% of Mn content (Supplementary Table 1).

For all the controls and treatments, Mn content was significantly higher in the landrace variety Lantvete than in the modern varieties KWS Bittern ($p = .001$) and Lennox ($p = .037$) (Supplementary Table 1 and Supplementary Figure 6).

2.1.7. Phosphorus

At baseline, P content of the wheat varieties varied from 488.84 to 540.63 mg/100g dw, it significantly increased with the different combination of climate scenarios treatments by 22.99–28.12% in Lantvete, 10.68–22.54% in Lennox, and 11.88–20.06% in KWS Bittern. Treatment A.O3 showed the highest P

content in KWS Bittern Lantvete, while in Lennox, it was treatment CT.O3. On the other hand, treatment C.EpO3 did not result in any change of P content in all the varieties (Figure 1G).

Treatments with chronic O₃ exposure, followed by episodic O₃, and then normal O₃, ambient CO₂ and higher temperature showed slightly higher values of P content. While interaction with O₃ exposure showed only little effect with high CO₂ and higher temperature, higher P content was obtained under chronic O₃ in the presence of ambient CO₂ and lower temperature (Supplementary Figure 7).

Submitting the wheat plants to water limitation lead to a significantly 10.85% lower P content in grain for treatment WLA.O3. As compared to control A, water limitation in combination with chronic O₃ exposure resulted in significantly higher P content of 7.13% and 17.80%, for treatments WLA.O3 and WLCT.O3, respectively (Supplementary Table 1).

In general, P content was significantly higher in variety Lantvete than in KWS Bittern ($p < .001$) and Lennox ($p < .001$) (Supplementary Table 1 and Supplementary Figure 7).

2.1.8. Zinc

The wheat varieties initially contained between 3.70 and 4.05 mg/100g dw of Zn, which drastically increased with the climate change treatments. Except treatment A.EpO3 which resulted in a non-significant Zn increase in KWS Bittern and Lantvete, all the other treatment heightened Zn content of the grains, by 35.35–73.52% in Lantvete, 18.66% to 69.29% in Lennox, and 14.24–48.15% in KWS Bittern. In all the varieties, the greatest Zn increase was recorded with treatments CT.EpO3 or CT.O3, followed by A.O3 and T.EpO3 (Figure 1H).

Higher contents of Zn were obtained with treatments with chronic O₃ exposure, followed by episodic O₃, and then normal O₃, high CO₂ and higher temperature. When combined, the two CO₂ treatments and the two temperature treatments were influenced by O₃ exposure: higher Zn content were obtained under chronic O₃, followed by episodic O₃, and then normal O₃ (Supplementary Figure 8).

While the effect of water-limited treatment showed a small (4.86%) reduction on Zn content, comparison with control A shows that combination of drought with other climate change factor significantly enhanced grain Zn by 46.21–40.95%, for treatments WLA.O3 and WLCT.O3, respectively (Supplementary Table 1).

Overall, variety Lantvete showed significantly higher Zn content than KWS Bittern ($p = .036$), but its content of Zn did not significantly differ from that of Lennox ($p = .097$) (Supplementary Table 1 and Supplementary Figure 8).

2.2. Effect of ozone

Ozone is one of the most damaging tropospheric air pollutant affecting plant growth and productivity^{28,37} and tropospheric O₃ concentrations have more than doubled since pre-industrial times³⁸. Wheat is sensitive to elevated O₃ levels, causing differences in grain yields and nutrient content¹³. In this study, treatments with O₃ alone did not have any noticeable change on C content, marginally reduced K content, but significantly increased the content of N (mean value for the 3 varieties, treatment A.EpO3 = 7.10% and treatment A.O3 = 33.22%), Fe (14.52% and 46.73%), Mg (12.94% and 25.24%), Mn (13.33% and 24.24%), P (9.29% and 20.85%), Zn (11.91% and 46.39%), protein (21.78% and 35.59%), and gluten (29.73% and 51.91%); chronic O₃ increased the content of the nutrients more than episodic O₃ (Figure 2).

2.3. Combined effects of climate change factors

It is realistic to consider that a combination of climate factors will simultaneously impact plant growth and production, and their combined effect on crop quality needs to be assessed. Interaction of O₃ with other abiotic factors on yield and nutrient content of plants has been very little considered. Comparison of treatments that combine elevated CO₂ and higher temperature with different O₃ regimes in this study (CT vs CT.O3 and CT.EpO3) showed that under condition of high CO₂ and temperature, O₃ could significantly increase grain contents of Fe (mean value for the 3 varieties, treatment CT.EpO3 = 11.86% and treatment CT.O3 = 21.32%), Mn (6.02% for CT.EpO3), P (3.37% for CT.O3 and Zn (15.71% for CT.EpO3 and 20.24% for CT.O3); the effect on the other minerals was not significant; chronic O₃ exposure was more effective than episodic (Figure 3). However, the effect of these combined climatic factors is not additive: comparing Figure 2 with Figure 3, it appears that the effect of O₃ on mineral content of wheat is greater in ambient CO₂ and lower temperature settings.

2.4. Yield effect

Because all the treatments caused decrease of yield²⁶, the resultant effect on nutrient yield (mass of grain nutrient per unit area) shows a correction towards significantly lower values, at various extend, depending on the initial content. In average, treatments A.EpO3 and WLA.O3 in KWS Bittern (median values of -10.52% and 9.89%, respectively) resulted in the highest reduction of nutrient content; in Lantvete, treatment A.EpO3 showed overall 33.84% reduction of nutrients; and in Lennox, treatment A.O3 resulted in 12.01% reduction (Figures 4A and 4B). As compared to the control, the strongest reductions due to yield were observed with K and C (median = 32.08% and 30.53%, respectively), followed by Mn (32.65%) and P (13.12%). The overall impact of climate treatments on nutrient availability was positive for gluten, Fe, Zn and protein, with an increase of 19.11%, 14.42%, 7.20% and 4.60%, respectively, while the decrease of yield counterbalanced the gain in concentration of the other nutrients, resulting in decrease of K (32.08%), Mn (21.65%), P (13.12%), and Mg (7.66%) (Figure 4C).

2.5. Trade-off between grain yield and protein content

The decline in protein content was significantly higher under future CO₂ conditions in comparison with the same plants grown with present-day CO₂. Moreover, yield explained a lower percentage of the variance in protein content under high CO₂. Hence, factors that are not related to atmospheric CO₂, such as genotypic differences between the cultivars may become increasingly important for the determination of protein content under future climate change conditions. Our results indicate that under present-day CO₂ level, protein content declines by 1.08% for 1 t/ha increase in yield, regardless of the treatment (low/high temperature, fully irrigated/water stressed, episodic/chronic exposure to O₃) or the wheat cultivar exposed (Figure 5).

2.6. Impact on nutrient intake and food security

Considering the content of nutrient in the control, the wheat consumption for European adults, and their average requirements (AR) of each nutrient, it was obtained that raw whole flour of the wheat varieties investigated in this study could potentially contribute to 45-52% of K needs, 75-95% for Zn and proteins, 93-120% for Mg and Fe, 265-293% for P, and 633-755% of Mn daily requirements (Figure 6A).

The different climate change treatments in this study resulted in contrasting effect on the percentage of dietary requirement potentially met by the intake of wheat: treatment A.EpO3 resulted in reduction of contribution to the daily AR of all the seven nutrients while treatments CT, CT.EpO3, CT.O3, T.EpO3 resulted in an increase, for the majority of the nutrients (Figure 6B). This effect of the climate change treatments was not significant among the three wheat varieties (Figure 6C). As a consequence, the climate change treatments resulted overall in 1.06- to 1.12-fold increase in raw whole wheat contribution in daily AR of Fe, Zn and protein, 1.03- to 1.06-fold decrease for Mg, Mn and P, and 2.78-fold decrease for K (Figure 6D).

3. Discussion

3.1. Basal mineral content of wheat varieties

As compared to published nutrient values for spring wheat, whole flour from the USDA database (NDB Number:20080) ² and of 176 spring wheat genotypes ³⁹, the values of controls in this study were lower for Fe, higher for K, Mn and P, and comparable for Mg, Zn and protein. Comparatively to the UK Composition of Foods Integrated Dataset (CoFID) 2021 ⁴⁰, the wheat varieties used in the current study contained 2-3 times more K, Mn, P, Zn and Mg, similar amount of protein, but lower Fe concentration than their counterpart (whole meal wheat flour, Food code: 11-889). The differences can be attributed to intrinsic parameters of the cultivars used in this study, and the different environment in which the wheat was grown. The interaction between genotype and the cultivation environment may play a significant role in the determination of the content and composition of wheat grains. For compounds like minerals and heavy metals, site specific variation are to a higher extent the determining factor, and the total content varies among years ⁴¹.

3.2. Effect of carbon dioxide

Current atmospheric CO₂ concentration (>411 ppm in 2019) are the highest since the last 800,000 years ⁴² and are predicted to increase to 550 ppm by 2050 ⁴³. Elevated CO₂ leads to lower content of Zn, Fe, Ca, S, N, protein and amino acids in wheat grain and other crop products ¹⁰⁻¹². For instance, a metanalysis showed that wheat grown under elevated CO₂ had significantly lower grain content of Zn, Fe and protein by 9.3%, 5.1% and 6.3%, respectively ¹⁰. More recently, it was found also that under elevated CO₂, concentrations of proteins, Zn and Fe in foliar and edible tissues of C₃ crops were reduced by 3-17% ²⁷. The mechanism by which high CO₂ reduces grain nutrient content is not yet clear. Different hypotheses have been proposed including decreased transpiration-driven mass flow of nitrogen ⁴⁴, carbohydrate dilution ⁴⁵, slow and reduced remobilization of N to the grain ⁴⁶, slower uptake of nitrogen in roots ⁴⁷ and inhibition of photorespiration and malate production ⁴⁸. Recent evidence suggest that CO₂ increase may have a “fertilization” effect on C₃ crops like wheat by increasing leaf photosynthesis in the absence of drought or elevated temperatures, leading to a decrease in the mineral concentration within the seeds ²⁷.

3.3. Effect of temperature

Since 1990, land surface temperature has increased by approx. 1°C due to anthropogenic increases in CO₂ and other greenhouse gases, and by the end of the century, it is projected that global mean surface temperatures will further increase by 2.6-4.8°C ⁴². Heat stress can affect wheat growth at any developmental stage, and it has a greater impact on grain yields and grain quality when the stress happens at the reproductive stage ^{9,49}. Post-anthesis heat stress (35°C) shortened the grain-filling duration and limited the allocation of resources to grains, leading to lower productivity by 6–51% in wheat plants grown in controlled environments and by 2–27% in plants grown under field conditions ⁵⁰. The mechanism underpinning these effect of heat stress are linked to its negative effects on floret initiation, including floral abnormalities (i.e. pistil hyperplasia and stamen hypoplasia) and pollen viability (i.e. microspore formation and pollen tube germination), leading to poor fertilization and reduced grain numbers ^{51,52}. Moreover, under heat stress, leaf senescence and photorespiration are accelerated, rubisco activity is reduced, photosystem II efficiency is disrupted, activities of starch-synthesizing enzyme are inhibited, reactive oxygen species are produced and their excessive level impair many cellular functions: all these result in reduction of the amounts of newly synthesized or stored assimilates that are translocated into developing grains ⁴⁹.

3.4. Effect of ozone

Our results on Figure 2 partially agree with other reports: elevated O₃ resulted in either reduced yield, sugar, starch and protein ¹³ or increase in K, Mg, Ca, P, Zn, Mn and Cu, and no effect on Fe, S and Na ¹⁴. In maize, chronic O₃ exposure decreased the content of grain P, Na, K, and increased Ca, Mg, Fe, and Cu ²⁸. The disparities can be explained by the high variability of sensitivity to tropospheric ozone among species and cultivars ^{13,53}. Ozone enters in the plant through the stomata of the underside of the leaf, and most likely reacts with molecules in the cell wall, and due to its strong oxidative property it triggers production of reactive oxygen species (ROS). The ROS damage cellular components, resulting in reduction of photosynthesis and other important physiological functions, acceleration of leaf senescence, and reduction of plant growth, with the consequence of weaker plants and impaired yield attributes ⁵⁴⁻⁵⁶. On the other hand,

O₃-stressed plants maintain to a larger extent N uptake while biomass accumulation is reduced, resulting in an increased grain protein content⁵⁷. Furthermore, increase of grain mineral concentration may be attributed to a more important synthesis and accumulation of these minerals, as a non-enzymatic antioxidant defense responses to abiotic stress⁵⁸. This could explain why plants under chronic O₃ exposure concentrated more minerals in their grain than the episodic O₃-exposed plant. The full molecular and physiological mechanism still needs to be elucidated.

3.5. Effect of drought

Climate predictions showed that towards the end of this century there will be a global increase in intensity and/or duration of drought, due to alternations in precipitation patterns and increased surface temperature, resulting in hotter and drier climate conditions⁴². In this study, when compared to their chronic O₃-treated counterparts, the water-limited treatments had no effect on C, Fe, Mg, increased Mn, and reduced N, K, P and Zn. On the other hand, when considered against the control treatment, the water-limited treatments under chronic O₃ exposure did not change the contents of C and K, and increased those of N, Fe, Mg, P, Mn, and Zn (Figure 1, Supplementary Table 1). This indicates that except for C, the effect of drought stress on wheat minerals is reverted under chronic O₃ exposure. Drought alone was reported to reduce protein and micronutrients in wheat^{18,19} and the content of grain N, K, Ca, Mn and Zn in maize⁵⁹. The degree of stomatal opening and its impact on plant photosynthesis depends on water status and other factors among which CO₂ availability²⁶. For adaptation of crops to future climate constraints, selection of cultivars resistant to heat and O₃ in association with irrigation have been recommended⁶⁰.

3.6. Combined effects of climate change factors

Our results show that overall, combinations of increased temperature, CO₂, O₃, and water deficit did not considerably influence grain C content of the wheat varieties, increased the concentration of N (by 33%), Fe (46%), Mg (23%), Mn (9%), P (16%), Zn (38%), protein (34%) and gluten (50%), and slightly reduced K content (-2%) (Figure 4C). Growth responses of wheat were affected differently by climate factor combinations, and change due to high temperature overrode the changes due to CO₂ and O₃²⁶. Combination of elevated CO₂ and high temperature can severely reduce duration of time to complete successive growth stages in wheat⁷. When temperature exceeds the optimum level for growth, the positive effects of elevated CO₂ on plant growth will be will reverted, as higher temperatures enhance stomatal closure, resulting in smaller CO₂ flux into leaves, and this decreases the level of photosynthesis⁶¹.

3.7. Varietal differences

In this study, except for K where KWS Bittern was dominant, the landrace variety Lantvete outperformed the others in content of all the nutrients, both for initial content and for response to climate treatments. Among organically grown wheat genotypes, landraces recorded higher content than cultivars, for twelve nutritionally important minerals³⁹. Lantvete showed grain yield plasticity across climate treatments, indicating a trend of not losing additional yields with the future climate scenario²⁶. Varietal diversity in response to elevated O₃ has been demonstrated in wheat^{13,62} and maize²⁸, and against heat stress in wheat⁹. Genotype and environment play a significant role in the determination of the content and composition of crop products⁴¹, and on primitive wheat, genotype had a higher impact than location³⁹. Variability of wheat cultivar responses to the combined effects of climate change factors is due to either more or less additive intraspecific variations and adaptations or it could be that one of the factors has an overriding influence on the impact of other factors²⁶. The variation of reduction of yield under elevated temperature across wheat cultivars in South Africa suggested that global warming impacts may be reduced through the sharing of gene pools among wheat breeding programs⁶³. These differences of response between cultivars offer a good opportunity for breeding towards more climate robust crops¹⁰, and our results suggest that Lantvete can be a good candidate for improved nutrients.

3.8. Yield effect

A previous report of meta-analysis of the effects of ozone on wheat quality demonstrated that O₃ improved the concentration grain protein and minerals (K, Mg, Ca, P, Zn, Mn, Cu), but the total amount per unit area was reduced, due to negative effect on yield¹⁴. Exposure of wheat cultivars in South Africa to temperatures above 30°C reduced wheat yield by 12.5%, and this heat effect was different across wheat cultivars⁶³. Combined effect of higher temperature and CO₂ was shown to reduce straw and grain yield in wheat⁷, as well as combination of elevated CO₂, O₃ and temperature reduced plant growth and yield of, oilseed rape and barley²¹. Important decrease of yield was obtained under the additive effect of water deficit and elevated O₃²⁵. The observed pattern of reduced yield and increased mineral concentration under O₃ exposure can be due to several factors. On one hand, O₃ is responsible for reduction of the duration of plant growth which shortens the period of nutrient uptake from the soil, reduction of root-to-shoot ratio because of reduction of photosynthesis and low amount of photosynthates available for root growth, and decrease of plant growth rate with consequently decreased nutrient uptake. On the other hand, stressed crops will try to maintain nitrogen uptake at the cost of biomass accumulation, resulting in enhanced protein and mineral concentration^{14,57}. To sum up, under climate change factors, the quality of wheat grain in term of nutrient concentration was improved, but the amount of protein and minerals accumulated per unit area was reduced, which may have serious implications for food security and human nutrition. Since yield reduction by climate change occurs when there is a need of more food to satisfy the demand of the global growing population to meet the demand of 9.1 billion people by 2050⁶⁴, it is important to assess how effect of climate of future decades on what could impact food security.

3.9. Trade-off between grain yield and protein content

Average decreases of 1.12% and 1.2% respectively in wheat grain protein content for 1 t/ha increase in grain yield were reported in previous studies^{65,66}. Our results are in line with the above studies. With regards to modelling the effect of O₃ on protein content, a simple statistical relationship between grain yield and protein content may provide an efficient parameterization. Under plant exposure to high O₃, the observed increase in protein content is a result of the decrease in grain yield⁶⁶, which is already simulated in some crop models⁶⁷⁻⁶⁹. It should be noted, however, that Eichi et al.⁷⁰ found that the relationship between grain protein content and yield for wheat in Australia varies between low and high productivity environments. Hence, the linear regression of Figure 5 may not be extrapolated below or above certain yield levels. Overall, our results agree with previous studies that the negative relationship between grain protein content

and yield becomes stronger under elevated CO₂^{71,72}, and a single linear regression based on yield may become less efficient in predicting protein content of wheat grain.

3.10. Impact on nutrient intake and food security

Considering the content of nutrient in the control, the obtained values of contribution to AR are very higher than another report which showed that consuming 160 g of commercially prepared whole-wheat bread contributes in meeting 36% of protein and 41% of minerals adequate intake (AI) of adults Europeans and Americans⁷³. Similarly, it was shown that consuming 200 g/day of whole wheat flour from 321 winter and spring wheat genotypes from the Nordic Gene Bank could meet 76% Fe needs of an adult German, 72-84% Mg, 78% Zn, 90% Mn, >100% P, and 41% K³⁹. The difference with our obtained results can be due not only to the location and environment effect on wheat nutrient content, but also on differences in reference intake values and nutrient losses during processing. In fact, there is an approximate 25% loss of protein, 90% loss of Mn, 85% loss of Zn, and 80% loss of Mg, K and Cu when wheat is milled and refined into flour⁷⁴, 1.7–4.6% loss of Cu, Mn, Fe and Zn during 60-day storage and baking of wheat flour⁷⁵. Intake of certain micronutrients are not met in many European countries: a study on adult nutrient intakes from national dietary surveys of European populations showed that although all countries met the female and male WHO recommended nutrient intakes (RNIs) for Zn, intakes of Fe, I and K were poorly attained in women⁷⁶. In the future, climate change could exacerbate or mitigate the nutrient availability in a largely consumed food like wheat and severely impact food security.

Given that no country in Europe met the RNI for K in women and only half of countries met the RNI in some male age groups⁷⁶, the recorded negative effect of climate change factors on K content in wheat could worsen the situation in future decades, resulting in increased risk of K deficiency. Between 1.5 to 2 billion people suffer from one or more chronic micronutrient deficiencies worldwide. If the current trajectory of 550 ppm atmospheric CO₂ by 2050 is maintained, the decrease of nutrients of C₃ crops under elevated CO₂ for example, could result in 175 million and 122 million more people to be Zn and protein deficient, respectively²⁷. These observations suggest that there is definitely an urgent need of climate-smart and nutrition-sensitive food systems, that could ensure that adaptation strategies to climate change and other disruptions take nutrition into account²⁹.

4. Conclusion

We evaluated the interactive effects of future climate scenario including higher temperature, elevated CO₂, different regimes of O₃, and water deficiency, on mineral content of three spring wheat varieties. We observed that plants under O₃ exposure alone concentrated more minerals, except K that was lower. Combined effect of O₃ in the presence of elevated CO₂ and higher temperature enhanced the content Fe, Mn, P and Zn. The effect of O₃ on mineral content of wheat was greater in ambient CO₂ and lower temperature settings, and was more pronounced under chronic O₃ exposure. The negative effect of drought stress on wheat minerals was reverted under chronic O₃ exposure, resulting in higher concentrations. The landrace variety Lantvete outperformed the others. This study confirmed previous models showing that under elevated CO₂, the negative relationship between grain protein content and yield becomes stronger, and demonstrated that a single linear regression based on yield would be less efficient in predicting wheat grain protein content. We analyzed the potential repercussions for food and nutrition security of these effects. Climate change could increase the contribution of wheat in meeting the daily nutrient requirements of Fe, Zn and protein, but decrease those of Mg, Mn, P and K. This may aggravate some nutrient deficiencies and hamper the reach of sustainable nutrition goal, or necessitate consumption of higher amount of wheat to reach the demand of some nutrients.

5. Methods

5.1. Plant materials

Three spring wheat varieties were studied, including two modern varieties (Lennox and KWS Bittern) and one landrace (Sweedish Lantvete). Lennox (Saaten-Union) used in southern France was supplied by Dr. Marie Launay, French National Institute for Agriculture, Food, and Environment (INRAE), Agroclim HDR, France. KWS Bittern used in Denmark was supplied by Danish Agro (Karise, Denmark). The landrace variety (Swedish Lantvete) was available from the Nordic Genetic Resource Center (NordGen), Swedish University of Agricultural Sciences, Alnarp, Sweden. All experiments were carried out according to institutional, national and international biosafety standards.

Their life cycle is between 3 to 4 months. Twelve seeds of each variety tested were sown in 11 L pots filled with 4 kg of sphagnum (Pindstrup Substrate No. 4, Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and thinned to 8 plants after germination, corresponding to ~165 plants/m². As the sphagnum was nutrient enriched with 10 g NPK fertilizer (21-3-10, Kemira Denmark A/S), no additional nutrients were added to the pots. Tap water was used for watering. Each variety was represented in each treatment with five pots.

5.2. Climate chamber

The experiment was performed in climate chambers that provided a controlled environment and uniform conditions, thus eliminating other potentially interacting parameters. The facility used was the RERAF phytotron (Riso Environmental Risk Assessment Facility, Technical University of Denmark, Riso, Denmark), which consists of six gastight chambers sized 6 × 4 × 3 m (length, width & height), providing detailed control of temperature, CO₂, air humidity, light, and O₃ concentration and exposure duration. Details of description of the climate chamber are available in^{21,77,78}.

5.3. Climate change treatments

Full details of the experimental conditions and treatments are available in Hansen et al.²⁶. Climate change treatments were selected among possible combinations of two present and future temperature levels (19/12°C or 24/17°C, both levels simulating days (16 h) that are warmer than nights (8 h)), two concentrations of CO₂ (400 and 700 ppm), and one of three O₃ regimes (no O₃ enrichment, episodic O₃ exposure, and full-time O₃ exposure). Ozone

concentrations for the treatments without O₃ enrichment was the climate chambers background levels (5.9 ± 0.5 to 7.2 ± 1.7 ppb), which are lower than the outside average O₃ concentration near the RERAF phytotron (average of 40.4 ppb, and maximum 1 h concentrations between 70.9 and 86.6 ppb). For both the episodic and full-time O₃ exposure treatments, O₃ concentration target was 80-100 ppb during the day (16 h of daytime O₃ exposure), and chamber background level equivalent to the no O₃ enrichment treatments at night. The full-time O₃ exposure treatments started at sowing, while the episodic O₃ exposure treatments began when Lennox variety reached Zadoks' developmental stage 31 (ZS31 - first node detectable) and ended when the variety reached stage 69 (ZS69 - anthesis complete)⁷⁹.

Throughout the experiment, relative humidity was maintained at 55/70% (day/night) for all treatments. To provide appropriate supply of water, plants were watered 3 times a week. All plants received increasingly more water as they grew, the warm treatment plants were, by design and by consumption, given more water than ambient treatment plants. Pots were weighed before and after watering to ensure the same amount of water was accessible in the treatment regardless of the pot's previous consumption. Additionally, a water-limited (WL) treatment was given to 2 selected climate combination treatments of variety KWS Bittern. It consisted of limited water supply in A.O3 and CT.O3, where the plants were subjected to chronic O₃ addition, in different CO₂ and temperature conditions. Thus, 10 climate treatment combinations were tested in total and named as follows:

- A = Ambient CO₂, lower temperature settings and no O₃ addition (control)
- A.EpO3 = Ambient CO₂, lower temperature settings and episodic O₃ addition
- A.O3 = Ambient CO₂, lower temperature settings and chronic O₃ addition
- C.EpO3 = High CO₂, lower temperature settings and episodic O₃ addition
- CT = High CO₂, higher temperature settings, and no O₃ addition
- CT.EpO3 = High CO₂, higher temperature settings and episodic O₃ addition
- CT.O3 = High CO₂, higher temperature settings and chronic O₃ addition
- T.EpO3 = Ambient CO₂, higher temperature and episodic O₃ addition
- WLA.O3: Ambient CO₂, lower temperature settings and chronic O₃ addition (i.e., A.O3), in water-limited condition
- WLCT.O3 = High CO₂, higher temperature settings and chronic O₃ addition (i.e., CT.O3), in water-limited condition.

Process values of treatment parameters such as relative humidity, CO₂ concentration, and temperature were logged by a data collection system several times per minute. The O₃ concentration was monitored twice every hour. At maturity, with moisture content around 9-13%, grains were harvested, threshed and winnowed, and the grains from plants of each replicate of treatment was mixed for further analyses.

5.4. Nutrient analysis

Grains were pulverized into whole wheat flour using a household blender, 600 mg of flour was weighed in a glass test tube, 3 mL of 69% HNO₃ (Hiperpur, Panreac, Spain) and 2 mL of deionized water (Milli-Q, Merck, Spain) were added. The mixture was digested in a microwave (Milestone, Ultrawave, Italy) at 240°C and 40 bar for 40 min at 1500 W, and the digesta was brought to a final volume of 50 mL with Milli-Q water. Minerals (C, N, F, K, Mg, Mn, P, and Zn) were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES). Analysis was performed on a PerkinElmer, Optima 4600 DV ICP-OES analyzer (Waltham, USA). The running parameters were set as follow: plasma flow 15 L/min, auxiliary flow 0.2 L/min, nebulizer flow 0.8 L/min, power 1300 W, reading distance 15 mm, reading position radial (K) and axial (Mg, Mn, Zn, Fe and P), integration time 5-10 s, and number of replicas 3. For quantification, standards (Panreac Química SLU, Spain) were prepared in HNO₃-H₂O in the same proportion as the samples (matrix matched calibration standards). Wheat standard reference material GBW10011 was used for recovery and limits determination. The detection and quantification validation parameters are summarized in Table 1. Nutrient content was corrected from grain moisture content, determined by using the Association of Analytical Communities (AOAC) Method 991.39⁸⁰. Content of N and C were expressed in g/100g dry weight (dw), while Fe, K, Mg, Mn, P and Zn were in mg/100g dw. Gluten content was determined according to the ICC 155 procedure⁸¹ by the Nordic Seed Laboratory Services, and expressed in percent of protein content. Protein content was obtained by multiplying the nitrogen content by 5.83⁸² and expressed in g/100g dw.

Table 1
Detection and quantification parameters of wheat minerals by ICP-OES.

Mineral	K	Mg	Mn	Zn	Fe	P
ICP-OES wavelengths (nm)	766.49	285.213	257.61	206.2	238.204	213.617
Standard concentration range	0.5-50	0.5-50	1-100	10-1000	10-1000	1-50
Standard concentration unit	mg/L	mg/L	µg/L	µg/L	µg/L	mg/L
Linearity	0.9999	0.9999	1.0000	0.9998	1.0000	0.9995
Recovery (%)	93.7	99.8	74.0	96.8	65.9	68.7
RSD (%)	6.4	6.5	5.9	7.6	6.1	6.2
LOD (mg/kg)	0.8111	0.0262	0.00276	0.0514	0.00178	2.604
LOQ (mg/kg)	36.198	0.173	0.00779	0.3098	0.2448	17.11

5.5. Impact on future food and nutrition security

To evaluate the overall effect of the treatments on grain nutrients availability, yield data from the experiment were obtained from Hansen et al.: all the treatments resulted in yield reduction, from 14–36% in KWS Bittern, 26–46% in Lantvete, and 16–37% in Lennox²⁶. Yield data of each treatment was used to correct the value of content of each nutrient, and the yield-corrected nutrient content were compared with the original ones. The potential repercussions of effect of both climate treatments and yield on food and nutrition security under future climate scenarios was analyzed with a case study of European adults. For this, per capita wheat consumption (298.55 g/day) was obtained from FAO food supply data for Europe¹. Daily average requirements (AR) of each nutrient were obtained from EFSA Dietary Reference Values for the EU database⁸³ for adults (≥ 18 years) males, and females not under any physiological status (pregnant, lactating, menopausal). For Zn, values at high level of phytate intake (LPI=1200 mg/day) were considered. These were combined with the yield-corrected nutrient content to estimate the potential percent contribution of each nutrient to average daily intake of some essential nutrients of adults in Europe.

5.6. Data analysis

Climate chamber experiments were performed in triplicate, and each wheat sample was analyzed in triplicate. Data were statistically analyzed using IBM SPSS Statistics v 26. ANOVAs were performed to determine the effect individual and combination of treatment on the content of each nutrient, one-way Dunnett's test with treatment A as control was applied for temperature, CO₂ and O₃ treatments. For drought experiment, each water-limited (WL) treatment was also compared against its corresponding control, i.e., WLCT.O3 vs CT.O3 and WLA.O3 vs A.O3. Additionally, performance of landrace variety Lantvete was compared with that of modern varieties KWS Bittern and Lennox using a 1-tailed pairwise Student's t-test. Similarly, a 1-tailed pairwise Student's t-test was also used to assess the significance of differences between original nutrient contents and yield-corrected nutrient contents. Three levels of significance (0.05, 0.01 and 0.001) were considered. The trade-off between grain yield and protein content was quantified using linear regressions of the three cultivars growing under baseline CO₂ (400 ppm) and future CO₂ (700ppm) levels; the grain yield data were converted to t/ha⁶⁹. Graphical representations were generated using R version R-4.0.1 and GraphPad Prism version 9.0.2 for Windows.

Declarations

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Author Contributions

EMØH & TNM conceptualized the crop study, devised the methodology and performed the crop chamber experiments. CO and AC conceptualized the nutritional aspects of climatic effects. YJHG, MJH & ID analyzed the data. YJHG & ID drafted the manuscript. All authors critically reviewed and approved the final manuscript. AC & TNM ensured the resources and funding for the crop project, CO and AC ensured the resources for the nutritional analysis and modelling work.

Competing Interests statement

The authors declare no competing interests.

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Figures

Figure 1

Effect of temperature, CO₂, ozone and water availability on grain mineral content of wheat. A = Ambient CO₂, lower temperature settings and no O₃ addition (control). A.EpO₃ = Ambient CO₂, lower temperature settings and episodic O₃ addition. A.O₃ = Ambient CO₂, lower temperature settings and chronic O₃ addition. C.EpO₃ = High CO₂, lower temperature settings and episodic O₃ addition. CT = High CO₂, higher temperature settings, and no O₃ addition. CT.EpO₃ = High CO₂, higher temperature settings and episodic O₃ addition. CT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition. T.EpO₃ = Ambient CO₂, higher temperature and episodic O₃ addition. WLA.O₃: Ambient CO₂, lower temperature settings and chronic O₃ addition (i.e., A.O₃), in water-limited condition. WLCT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition (i.e., CT.O₃), in water-limited condition. ns, *, ** and *** mean non-significant, significant at 0.05, 0.01 and 0.001, respectively, Dunnett's test with treatment A as control.

Figure 2

Change of mineral content of three European wheat varieties under effect of ozone. A = Ambient CO₂, lower temperature settings and no O₃ addition (control). A.EpO₃ = Ambient CO₂, lower temperature settings and episodic O₃ addition. A.O₃ = Ambient CO₂, lower temperature settings and chronic O₃ addition.

Figure 3

Change of mineral content of three European wheat varieties under combined effect of different ozone regimes in condition of high carbon dioxide and elevated temperature. CT = High CO₂, higher temperature settings, and no O₃ addition. CT.EpO₃ = High CO₂, higher temperature settings and episodic O₃ addition. CT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition.

Figure 4

Changes in grain mineral content of three European wheat varieties under climate change treatments before (A) and after (B) yield correction, and overall comparison of effect on each nutrient (C). A = Ambient CO₂, lower temperature settings and no O₃ addition (control). A.EpO₃ = Ambient CO₂, lower temperature settings and episodic O₃ addition. A.O₃ = Ambient CO₂, lower temperature settings and chronic O₃ addition. C.EpO₃ = High CO₂, lower temperature settings and episodic O₃ addition. CT = High CO₂, higher temperature settings, and no O₃ addition. CT.EpO₃ = High CO₂, higher temperature settings and episodic O₃ addition. CT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition. T.EpO₃ = Ambient CO₂, higher temperature and episodic O₃ addition. WLA.O₃: Ambient CO₂, lower temperature settings and chronic O₃ addition (i.e., A.O₃), in water-limited condition. WLCT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition (i.e., CT.O₃), in water-limited condition. *** indicates significant difference at 0.001 between the normal and the yield-corrected nutrient content, one-tailed pairwise Student's t-Test.

Figure 5

Relationship between grain yield and protein content for spring wheat varieties KWS Bittern, Lennox and Lantvete grown under baseline CO_2 (400 ppm; red points) and high CO_2 level (700 ppm; blue points). Solid red and blue lines are linear regressions fitted against the red and blue points respectively and grey areas are 95% confidence intervals.

Figure 6

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Contribution of wheat raw whole flour consumption of 298.5 g/person/day to daily average requirement of some essential nutrients of European adults (≥ 18 years): for 3 spring wheat varieties (A), effect of climate change treatments (B), effect of treatments on each variety (C) and overall effect of climate change factors on each nutrient (D). A = Ambient CO₂, lower temperature settings and no O₃ addition (control). A.EpO₃ = Ambient CO₂, lower temperature settings and episodic O₃ addition. A.O₃ = Ambient CO₂, lower temperature settings and chronic O₃ addition. C.EpO₃ = High CO₂, lower temperature settings and episodic O₃ addition. CT = High CO₂, higher temperature settings, and no O₃ addition. CT.EpO₃ = High CO₂, higher temperature settings and episodic O₃ addition. CT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition. T.EpO₃ = Ambient CO₂, higher temperature and episodic O₃ addition. WLA.O₃: Ambient CO₂, lower temperature settings and chronic O₃ addition (i.e., A.O₃), in water-limited condition. WLCT.O₃ = High CO₂, higher temperature settings and chronic O₃ addition (i.e., CT.O₃), in water-limited condition. *** indicates significant difference at 0.001 between the normal and the yield-corrected nutrient content, one-tailed pairwise Student's t-Test.

Supplementary Files

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